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Motor simulation of emotional facial expressions in autism

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Age-related increase in inferior frontal gyrus activity and social functioning in autism (2011)
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Abstract

Background Hypoactivation of the inferior frontal gyrus during the perception of facial expressions has been interpreted as evidence for a deficit of the mirror neuron system in children with autism. We examined whether this dysfunction persists in adulthood, and how brain activity in the mirror neuron system relates to social functioning outside the laboratory.

Methods Twenty-one adult males with Autism Spectrum Disorder and 21 typically developing subjects matched for age, gender, and IQ were scanned in three conditions: observing short movies showing facial expressions, performing a facial movement, and experiencing a disgusting taste. Symptom severity and level of social adjustment were measured with the Autism Diagnostic Observation Schedule and the Social Functioning Scale.

Results Inferior frontal gyrus activity during the observation of facial expressions increased with age in autism, but not in controls. The age-related increase in activity was associated with changes in gaze behavior, and improvements in social functioning. These age-related neurocognitive improvements were not found in a group of individuals with schizophrenia, who had comparable levels of social functioning.

Conclusions The results of this cross-sectional study suggest that mirror neuron system activity augments with age in autism and that this is accompanied by changes in gaze behavior and improved social functioning. It is the first demonstration of an age-related neurocognitive improvement in autism. Increased motor simulation may contribute to the amelioration in social functioning documented in adolescence and adulthood. This finding should encourage the development of new therapeutic interventions directed at emotion simulation.

4.1 Introduction

Autism is a lifelong disorder defined by impairments in social and communicative functioning and by pronounced behavioral rigidities (Lord, Cook, Leventhal, & Amaral, 2000; F. R. Volkmar, Lord, Bailey, Schultz, & Klin, 2004). Autism Spectrum Disorder (ASD) have a strong genetic component, but no biological marker is available to date. An influential (Iacoboni & Dapretto, 2006; Rizzolatti & Fabbri-Destro, 2008; Rizzolatti et al., 2009; Williams et al., 2001) but controversial (Dinstein et al., 2008; Southgate & Hamilton, 2008) theory holds that the core social difficulties in ASD originate from a dysfunction of the putative mirror neuron system (MNS). Mirror neurons are found in macaques, in ventral premotor and inferior parietal regions involved in action execution. Single-cell recordings demonstrate that these neurons fire when the monkeys perform an action, and when they observe a similar action (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Fogassi et al., 2005; Fujii, Hihara, & Iriki, 2008; Gallese et al., 1996). The discovery of this mirroring property challenges the distinction between action and perception and suggests motor programs may play a role in action understanding (Rizzolatti et al., 2001). A subset of ventral premotor neurons triggering mouth actions also fire to the observation of similar mouth actions, including communicative gestures (Ferrari et al., 2003). Single-cell (Mukamel et al.), fMRI (Buccino et al., 2001; Filimon et al., 2007; Gazzola et al., 2007; Grèzes et al., 2003) and TMS (Fadiga et al., 1995; Urgesi, Moro, Candidi, & Aglioti, 2006) studies show that a similar system exists in humans. The motor simulation mechanism implemented in the human MNS may contribute to understanding the intentions behind the actions of others (Rizzolatti & Craighero, 2004). This also seems to be true for emotional facial expressions (Bastiaansen, Thioux, & Keysers, 2009), which trigger an increase of activity in the precentral motor face area of the observer (Carr et al., 2003; Fox, Iaria, & Barton, 2009; Leslie et al., 2004; Schilbach et al., 2008; van der Gaag et al., 2007; Wicker et al., 2003) that is associated with facial mimicry (Schilbach, Eickhoff, Mojzisch, & Vogeley, 2008). The observer (unconsciously) mimics the emotion in a muscle-specific manner (Dimberg, 1982, 1990; Dimberg et al., 2000), which can facilitate emotion recognition (Niedenthal, 2007; Niedenthal et al., 2001; Oberman et al., 2007). Adopting emotion-specific postures triggers the corresponding emotion (Strack et al., 1988), while motor interference modifies the subjective experience of observed emotions (Effron et al., 2006). The interaction between emotion perception and motor simulation might be instantiated by the inferior frontal gyrus (IFG: Brodmann's area [BA] 44/45) and the anterior insular cortex (Carr et al., 2003; Jabbi et al., 2007), which are anatomically connected (Nanetti, Cerliani, Gazzola, Renken, & Keysers, 2009). The anterior insular cortex, thought to represent bodily sensations (Craig, 2002), may serve as a relay between the premotor cortex and the limbic system (Carr et al., 2003; Dapretto et al., 2006; Jabbi et al., 2007). Activity in the IFG during the perception of a disgusted expression indeed seems to cause increased activity in the anterior insular cortex (Jabbi & Keysers, 2008). High empathizers activate these regions more strongly (Jabbi et al., 2007; Pfeifer et al., 2008) and mimic more (Sonnby-Borgstrom, 2002), which underlines the importance of motor simulation for emotion recognition and empathy (Bastiaansen et al., 2009).

In this context, the finding that children and adolescents with ASD fail to activate the IFG normally during the perception and the imitation of emotional facial expressions (Bookheimer, Wang, Scott, Sigman, & Dapretto, 2008; Dapretto et al., 2006; Greimel et al., 2010; Uddin et al., 2008) suggests a MNS dysfunction that can potentially impact social comprehension. The first

experiment tested children of 12 ± 2 years of age, and found a significant (negative) correlation between IFG activity and symptom severity (Dapretto et al., 2006). In fact, at the group level children with ASD did not show any significant IFG activity during the observation of emotional facial expressions. Three subsequent investigations with children and adolescents produced similar findings in tasks where the subjects had to match upright and inverted faces (Bookheimer et al., 2008), had to recognize themselves on a set of morphed pictures (Uddin et al., 2008), or had to judge their own emotional response while empathizing with a face on the screen (Greimel et al., 2010). Previous investigations with adults have provided mixed results, with two out of three studies failing to show significant group differences in the IFG for face perception (Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007; Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2007; Pierce, Haist, Sedaghat, & Courchesne, 2004). However, the sample size in these studies was small (approximately 10 subjects per group), and groups were not matched on critical variables. Here, using fMRI and dynamic facial expressions, we examine the relationship between IFG activity, autistic symptoms, and social behavior outside the laboratory in an adult population of 21 males with ASD that are pair-matched on age and IQ with 21 typically developing males.

4.2 Methods and Materials

4.2.1 Participants

Twenty-one adult males with ASD (age M=30.6, SD=10.09, range=18-54 years) were recruited via local mental health institutions and through mailing lists. All subjects were diagnosed with autism, Asperger Syndrome, or PDD-NOS by a clinical psychologist or psychiatrist according to DSM-IV-TR criteria (American Psychiatric Association, APA, 2000). Clinical diagnoses were verified with the Autism Diagnostic Observation Schedule (ADOS, Lord et al., 2000). One of the subjects scored below the communication domain cut-off; his diagnosis was confirmed by the Autism Diagnostic Interview Revised (ADI-R, Rutter, Le Couteur, & Lord, 2003). The subjects were considered to be high-functioning by their clinicians and none had an IQ score below 70 (IQ M=102.5, SD=14.81) on the Groninger Intelligence Test 2 (GIT2, Luteijn & Barelds, 2004). The control group consisted of 21 typically developing males (age M=30.5, SD=9.85, range=18-53 years) that were pair-matched on age and IQ (IQ M=101.5, SD=17.40) with the subjects in the ASD group (Supplementary Table 1). The presence of major psychiatric disorders was ruled out by the administration of the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry (SCAN 2.1, Giel & Nienhuis, 1996). In addition, they were interviewed to verify that first-degree relatives did not have a pervasive developmental disorder or a history of psychosis. All subjects had normal or corrected-to-normal hearing and vision, were eligible for MRI research, and gave written informed consent to participate in the study, which was approved by the Institutional Review Board of the University Medical Center Groningen (METc).

4.2.2 Behavioral Measures

We assessed each subject's current level of social adjustment through the Social Functioning Scale (SFS, Birchwood, Smith, Cochrane, Wetton, & Copestake, 1990), which has originally been

developed for schizophrenia. The SFS, which is filled out by both the subject (SFS-client) and an informant (e.g. a parent, SFS-other), is preeminently a measure of current social adjustment in people with known social difficulties, because it is a continuous measure that taps those areas that are crucial to community maintenance (e.g. prosocial activities, independent living skills, employment). For the ASD group, we additionally used the social domain of the ADOS as a measure of symptom severity.

4.2.3 FMRI Tasks

The study of mirror mechanisms not only requires measuring brain activity when subjects perceive, for instance, the emotion of another individual, but also when they themselves feel or express an emotion. Therefore, subjects first performed an observation task, followed by two control tasks: facial movement execution, and emotion experience through a disgusting taste (see Supplementary Methods).

Observation of dynamic facial expressions

The observation task comprised two visual runs, during which subjects were asked to carefully watch short movies of facial expressions (3 s, 14° x 18°). Each run consisted of the same 60 movies presented in random order, which showed (a) actors making a disgusted, pleased or neutral facial expression (i.e. blowing up the cheeks) or (b) actors responding as naturally as possible to one of three tastes: water (neutral condition), lemon juice (disgust condition) or a sweet juice (pleasure condition). In these cases, the actors responded with a clear emotional facial expression after tasting the liquid through a straw (Figure 1a). For each stimulus type there were eight different actors (male/female), who were recruited from a local professional theatre company and a youth theatre school. The movies were validated and used in two previous experiments (Jabbi et al., 2007; van der Gaag et al., 2007). Movies were separated by a red fixation cross (1° x 1°) with an intertrial interval that varied randomly between 5 and 12 s (baseline). A still image of the background against which the actors were filmed was presented at the beginning of each run and served as a background for the fixation crosses to improve stability of the eye tracking signal (see Supplementary Methods) by keeping the pupil size constant. Stimuli were presented using Presentation software (Neurobehavioral Systems Inc., Albany, CA, USA).

4.2.3 Magnetic Resonance Images acquisition and preprocessing

See Supplementary Methods.

4.2.4 fMRI Analysis

Subject level analysis

Statistical analyses were implemented using the Statistical Parametric Mapping software package (SPM2, Wellcome Department of Cognitive Neurology, London, UK: http://www.fil.ion.ucl.ac.uk) and region of interest (ROI) toolbox MarsBaR (http://marsbar.sourceforge.net/). Time series were

high-pass filtered at 385s for the visual runs to remove low-frequency noise and slow drifts in the signal. At the subject-level, separate predictors were used as boxcar functions convolved with the hemodynamic response function for the six movie types (disgust, pleasure, neutral either with or without a cup).

Group analyses

Dapretto and colleagues (Dapretto et al., 2006) found that the strongest difference between children with autism and typically developing (TD) children during the imitation of facial expressions was located in the pars opercularis of the right IFG (BA44) around peak coordinates (57, 10, 16). To examine activity in this region (Figure 1b) in adults, we first created a spherical ROI centred on the corresponding MNI coordinates (Figure 1c). In the absence of information on the cluster size of the activated region, we used a 5 mm radius sphere for our analyses. We checked whether our ROI had mirror properties (Figure 1c) by examining its activity in the control group during facial expression execution and during emotion experience (see Supplementary Methods). Next, contrast estimates for all six movie types were extracted from the ROI at the subject level and subjected to a Mixed Model ANCOVA with factors Emotion x Context x Group, including IQ and Age as covariates. Because the effect of group did not interact with factors Context or Emotion, we then averaged the contrast estimates per subject over emotion and context to compute the general effect of watching facial expressions. Subsequently, we set up a multiple regression analysis in MarsBaR with six columns in the design matrix: one constant for each group, and separate IQ and age covariates for each group to account for the broad age and IQ range in both groups (18-54 yrs, 73-133 IQ pts). This analysis was repeated after the removal of two outliers, whose BA44 activity was more than two standard deviations apart from the group mean. To explore whether the effects found in the ROI were spatially limited, we repeated this analysis for all voxels in the brain using SPM (without removing the outliers).

To examine whether there was hypoactivity in the ROI for the younger subjects with ASD, we selected the eight youngest and eight oldest subjects of each group and ran two independent sample *t* tests. A sample size of eight is enough to enable parametric statistics, while preserving sufficient difference in age between the subgroups.

Large variability in brain responses to a stimulus reduces the information that a region can provide about that stimulus. It has recently been proposed that in ASD premotor regions show more variable responses to the vision of action (Dinstein et al.), which challenges their contribution to social perception. To examine whether premotor responses were less consistent in the ASD group during facial expression observation, we calculated in our ROI each subject's correlation between the modeled and measured time courses across the two perception runs. The correlations were analyzed across participants using a multiple regression analysis in MarsBaR with a single entry per participant, separate constants for the ASD and TD groups, and covariates for IQ and age.

Finally, since in children with ASD BA44 activity predicts symptom severity (Dapretto et al., 2006) and social competence (Pfeifer et al., 2008), we examined the link between social symptoms, social adjustment, and brain activity in adults with ASD. To this end, we calculated the linear pairwise regressions of BA44 activity, age, ADOS (social domain) and SFS scores, and compared the regression slopes with those of the control group if applicable and with those of a group of

participants with a diagnosis of schizophrenia (see Supplementary Methods). The variability in SFS scores was too low in the TD group to perform regression analyses.

4.3 Results

4.3.1 Behavioral Measures

Social Functioning Scale

SFS scores were significantly lower in the ASD group compared to the TD group (SFS-client: T(22.8)=-6.234, *p*=.000, SFS-other: T(23.4)=-7.205, *p*=.000). The variability in SFS scores was very low in the TD group, reflecting a ceiling effect (TD: σ^2=9, ASD: σ^2=107; *p*<.005).

Movie ratings

The ratings collected after scanning for the different emotions are summarized in Figure S1.

4.3.2 fMRI analysis

fMRI group comparison

During the observation of dynamic emotional facial expressions, high-functioning adults with ASD activated a similar neural network as TD subjects, including BA44 (Supplementary Tables 2 and 3). Compared to the TD group, the ASD group did not show reduced activity in any region of the brain using a standard threshold (T=3.33, uncorrected *p*=.001, k=20, Figure S2), nor was there any group difference in the BA44 ROI (one-tailed T(40)=1.16, *p*=.13). To further examine if there is an effect of emotion (Disgust, Neutral vs. Pleasure) and/or the presence of a context (Cup vs. No Cup) on the group difference, we analyzed the signal measured in these six conditions in the ROI using an Emotion (3) x Context (2) x Group (2) Mixed Model ANCOVA including IQ and age as nuisance variables. This analysis confirmed the absence of a main effect of Group (F(1,38)=1.07, *p*=.31) and found no evidence for interactions of Group x Emotion (F(2,37)=1.66, *p*=.20), Group x Context (F(1,38)=.78, *p*=.38) or Group x Emotion x Context (F(2,37)=1.41, *p*=.25). Accordingly, we examined the average activity across all six stimulus types in all further analyses.

Age effect on brain activity

The regression analysis in the predefined BA44 ROI suggests that age may be a critical factor in determining BA44 activation: while there was no main effect of Age (F=.33, *p*=.57), nor IQ (F=.03, *p*=.87), there was a significant interaction for IQ x Group (F=4.73, *p*=.04), and a highly significant interaction for Age x Group (F=10.55, *p*=.003). After the removal of two outliers (see methods), the Age x Group interaction became even more significant (F=15.93, *p*=.000), while the interaction of IQ x Group disappeared (F=2.51, *p*=.13). As shown in Figure 1d and Figure 2a-b, activity in BA44 during emotion perception increased with age for the ASD group (n=19, slope=3.1, T=2.89, *p*=.003), but not for the TD group (n=21, slope=-2.9, T=-2.75, *p*=.99) with the slopes being significantly different (*p*=.003). The whole-brain analysis did not reveal any regions showing a significant IQ x Group

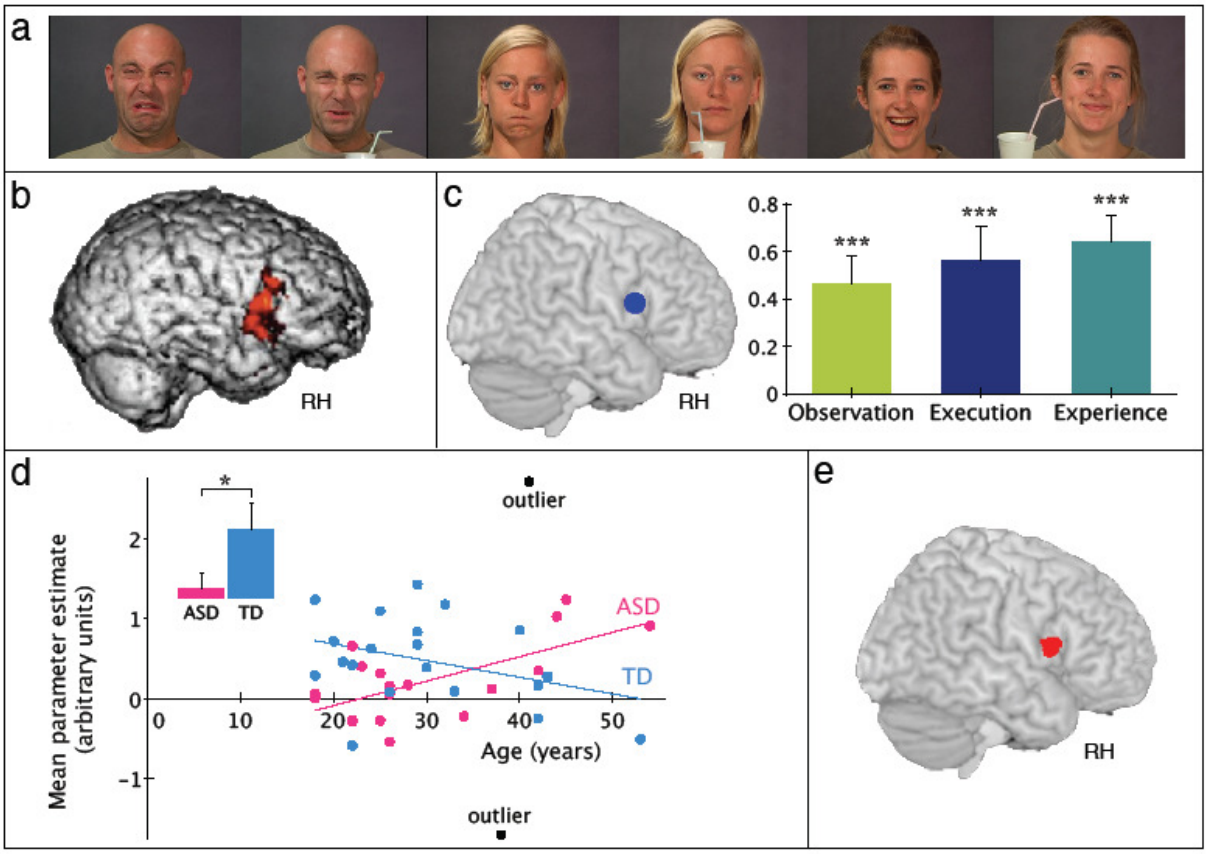
interaction. In contrast, the Age x Group was significant in one single region of the brain: right BA44 (Talairach coordinates: 58, 12, 12), which matches the area of hypoactivation in children with ASD perfectly (Fig. 1e). Selection of the eight youngest subjects in each group showed that young adults with ASD (BA44 M=.01, age M=21.9, IQ M=93.6) activated the BA44 ROI significantly less than their TD peers (Figure 1d, BA44 M=.53, age M=21.3, IQ M=89.9, $F(1,13)=6.16$, $p=.03$). For the oldest subjects there was no significant difference between the groups, $F(1,13)=.47$, $p=.51$.

If a group has higher variability in brain response, the predicted brain response (i.e. time course of the task convolved with the hemodynamic response) should correlate less with the measured brain response. We found no significant difference in this correlation in our BA44 ROI between the ASD and TD group (one-tailed T=-.37, $p=.64$). However, there was a differential effect of age in the two groups ($F=6.99$, $p<.01$): the correlation increased (i.e. unexplained variance decreased) with age in the ASD group (slope=.3, T=2.41, $p=.01$), but not in the TD group (slope=-.2, T=-1.34, $p=.91$).

Social functioning and BA44 activity

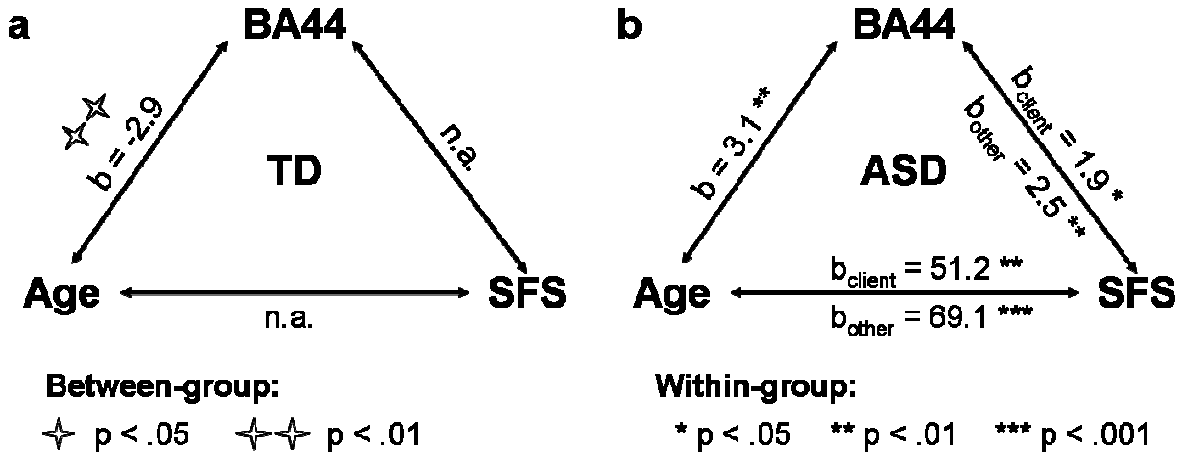
To examine the behavioral significance of our findings, we investigated the relationship between age, BA44 activity, a measure of autistic symptoms (ADOS social domain), and a measure of social adjustment (SFS) that assesses the subject's engagement in activities that are crucial to community maintenance. In the ASD group, age, BA44 activity, and SFS scores were significantly and positively associated (Figure 2): older subjects not only activated BA44 more, they were also more socially adjusted than younger individuals. In contrast, the social domain of the ADOS was not significantly correlated with age, BA44 activity, nor SFS scores (all $p>.24$), suggesting that age-related changes in BA44 activity were associated with social adjustment as measured using the SFS, but not with the remission of autistic symptoms as measured using the ADOS. Because the TD group is characterized by high social functioning and little variation in SFS scores, we cannot assess whether the link between age and SFS and between IFG activity and SFS is specific to autism or whether it would be observed in any population with social functioning deficits. To disentangle these possibilities, we tested a group of individuals with schizophrenia having predominantly negative symptoms, which are frequently associated with social deficits (Uta Frith & Happé, 2005) and autistic-like symptoms (Bastiaansen et al., 2011b; Sheitman et al., 2004) in schizophrenia. These analyses demonstrate that age-related increases in BA44 activity and social functioning seen in ASD, do not occur in schizophrenia (see Supplementary Methods and Supplementary Figure 3).

Figure 1 ROI definition, stimuli and fMRI results



(a) Still frames at maximum intensity of the disgusted, neutral, and happy facial expressions with and without the presence of a gustatory stimulus. Neutral movies involved movement of the face to make them more comparable to the emotional facial expressions (third from left: blowing up of the cheeks, fourth from the left: tasting and lip movements). (b) Hypoactivation found in children with ASD (Dapretto et al., 2006). c) MNS ROI (5 mm sphere) based on peak coordinates in right BA44 where a group difference was reported in children (MNI: 56, 10, 14). In the TD group the ROI was not only significantly active during the observation of emotional facial expressions, but also during the execution of a facial expression, and during emotional experience (*** = uncorrected p<.001). This suggests that the ROI in our sample of TDs has mirror properties. (d) Scatter-plot of the Age x Group interaction in the BA44 ROI: the older the subjects with ASD (pink), the stronger the activity and vice versa for the TDs (blue). The bar graph in the top left shows the activity in BA44 for the youngest adults with ASD (pink, n=8, age M=21.9) compared to the youngest TDs (blue, n=8, age M=21.3), p<.05. (e) The whole-brain analysis showed that the interaction between age and group is maximal in BA44 (k=94).

Figure 2 Regression diagram



The diagrams show linear pairwise regressions between BA44 activity, age and social functioning (if applicable) in the (a) TD group, and (b) ASD group. Regression slopes b are expressed in arbitrary units (x 100) per year/ SFS point and are reported in combination with their respective significance levels (* p<.05, ** p<.01, *** p<.001). Regression slopes that are significantly different from the ASD group are marked by stars in panel (a). SFS-client refers to the questionnaire that was filled out by the subject, SFS-other refers to the version that was filled out by an informant (e.g. parent or caretaker).

4.4 Discussion

In this cross-sectional study, we measured brain activity during the observation of dynamic facial expressions in a group of adults with Autism Spectrum Disorder compared to pair-matched controls. While three previous investigations with children around 12 years of age had consistently found significant hypoactivity of the IFG (Bookheimer et al., 2008; Dapretto et al., 2006; Uddin et al., 2008), in our relatively large sample of adults both groups activated this location to the same extent, even when the analysis was restricted to the region of hypoactivity in children. This confirms the results of two other studies reporting whole-brain analyses for an adult population, in which no group difference was found involving the IFG (Ashwin et al., 2007; but see Hadjikhani et al., 2007; Pierce et al., 2004). The discrepancy between findings in children and adults is intriguing. Our study demonstrates that age might be a critical factor determining IFG activity in ASD: activity increased with age in the autism group but not in the control group, so that by age 30, individuals with ASD no longer differed from typically developing individuals. In addition, the within-subject variance decreased with age in the ASD group. This suggests that neural 'noise' in the IFG (Dinstein et al., 2010) decreases with age in ASD, which may be indicative of improved functioning of the MNS. Our results suggest prima facie that motor simulation of facial expressions follows a developmental trajectory with a deficit affecting individuals with ASD during their first years of life, and vanishing somewhere in late adolescence or early adulthood. Importantly, we found that the age-related increase of activity in pars opercularis of the IFG (BA44) in ASD was associated with improvements in social functioning. Increased simulation of facial expressions in the IFG is likely to affect emotion recognition, and enhance the ability of some adults with ASD to share the feelings of others (see introduction). This probably has a positive impact on social affiliation, and plays a

positive role in the construction of a tissue of social relationships (Lakin, Chartrand, & Arkin, 2008; Lakin, Jefferis, Cheng, & Chartrand, 2003). It is well-established that there is a certain degree of abatement in the behavioral difficulties experienced by individuals with ASD throughout adolescence and adulthood (Farley et al., 2009; McGovern & Sigman, 2005; Piven, Harper, Palmer, & Arndt, 1996; Seltzer et al., 2003; Shattuck et al., 2007). Improvements are mostly seen in high-functioning individuals (Howlin, Goode, Hutton, & Rutter, 2004; Shattuck et al., 2007) and concern social behaviors, as well as language, repetitive/stereotyped behaviors, and emotional responsiveness to other's distress. Here, we found an improvement of social functioning (as measured using the SFS) with age, while autistic symptoms (as measured using the ADOS) did not change significantly. This could be the consequence of a selection bias, since we selected only participants who scored on the ADOS. Alternatively, it could indicate that while autistic symptoms predominantly persist, the way individuals with ASD cope with their social difficulties improves with age. Although speculative, this would be consistent with a longitudinal study showing that age significantly predicts a decline of maladaptive behaviors such as withdrawal and inattentiveness, but not of autistic symptoms (Shattuck et al., 2007). The relationship between IFG activity and social functioning could not be investigated in typically developing individuals, because they showed little variation in scores on the social functioning measure. To examine whether the association between IFG activity, age, and social functioning was specific to autism, we included a group of individuals with schizophrenia (see Supplementary Methods). Although the scores on the social functioning scale were comparable to those in the ASD group, IFG activity was not associated with age or social functioning in schizophrenia, suggesting that the developmental pattern might be unique to ASD. Here, it is important to note that the SFS has not been age-normed in an older adult population (>30 yrs). However, the fact that we found no evidence of increased SFS scores with age in individuals with schizophrenia argues against the idea that the age-related increase of SFS scores in the ASD group reflects an inherent property of the SFS measure. Instead, our findings are compatible with the idea that increased motor simulation could lead to the documented age-related improvements in social functioning in autism and the improved responsiveness to other's distress evidenced throughout adolescence (McGovern & Sigman, 2005).

Further research is needed to determine the origin of the increased activity in the IFG during face perception, but the analyses conducted on the available eye tracking data (see Supplementary Methods) suggest that eye gaze behavior might be determinant (Corden, Chilvers, & Skuse, 2008; Dalton, Nacewicz, Alexander, & Davidson, 2007; Dalton et al., 2005; Senju et al., 2009; Spezio et al., 2007a; Vivanti, Nadig, Ozonoff, & Rogers, 2008). On a group level, eye gaze behaviors in our study did not differ between the individuals with ASD and TDs. This could mean that motor simulation is normal in adults with ASD –as long as they pay attention to the same aspects of the face as controls (the same conclusion has also been reached for the fusiform gyrus during face processing: Dalton et al., 2005). Again, age seems to play a critical role. In normal ageing, time spent looking at the eyes decreases, while fixations to the lower part of the face increase (Sullivan, Ruffman, & Hutton, 2007; Wong, Cronin-Golomb, & Neargarder, 2005). Here, we found that in adults with ASD the amount of time spent on the lower half of the face also increases with age. The associated increase in BA44 activity suggests that this could be a beneficial strategy for individuals with ASD. Individuals with ASD reach higher levels of accuracy on emotion recognition (Spezio et al., 2007a) and familiar face recognition tasks (Langdell, 1978) when presented with information from

the lower regions of the face, particularly the mouth region, compared to the eye region. Perhaps high-functioning individuals with ASD, while growing older, learn to look more at the parts of the face that are most relevant for them to decode emotional facial expressions. Further research is needed to investigate this hypothesis and its implications. For instance, increased fixations to the lower part of the face might lead to better recognition of some (e.g. disgust), but not other (e.g. fear) emotions not tested in this experiment (Wong et al., 2005).

If there is an actual improvement of facial simulation abilities in ASD during adolescence and early adulthood (Beall, Moody, McIntosh, Hepburn, & Reed, 2008; Magnée, De Gelder, van Engeland, & Kemner, 2007), and if it contributes to social adjustment as suggested by our study, therapeutic interventions targeting the same mechanism should be experimentally tested in children. Some recently developed training methods produce significant improvements for face recognition (Tanaka et al.) as well as emotion recognition (Golan et al., 2009; Golan & Baron-Cohen, 2006), but the generalization from training material to real-life is not guaranteed (Golan et al., 2009; Golan & Baron-Cohen, 2006). We are not aware of any study reporting the effect of training motor simulation of facial expressions. The MNS is flexible, and learning is possible even in adulthood (Catmur et al., 2007; Lahav, Saltzman, & Schlaug, 2007). Furthermore, expertise in a motor domain is associated with increased activity in the MNS during the observation of similar movements (Calvo-Merino et al., 2005; Cross, Hamilton, & Grafton, 2006; Haslinger et al., 2005). Therefore, children with ASD might particularly benefit from imitation training.

In conclusion, activity in the IFG during the perception of dynamic facial expressions increases with age in autism, and this is associated with improved social functioning. It is the first published evidence of an age-related neurocognitive improvement in autism, and suggests that individuals with ASD may learn to improve their social skills over the course of life. There was no significant age-related change in a group of individuals with schizophrenia with comparable levels of social functioning, suggesting that our findings might be specific to autism. Because autism is a developmental pathology with changes occurring over the lifetime, researchers should examine how individuals with ASD develop to deal with their initial dysfunctions, and how therapeutic interventions aimed at promoting motor simulation of emotional expressions can support this process.

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4.5 Supplementary Methods

4.5.1 fMRI Control Tasks

The study of mirror mechanisms not only requires measuring brain activity when subjects perceive, for instance, the emotion of another individual, but also when they themselves feel or express an emotion. Therefore, the fMRI study consisted of three tasks: observation of short movies showing emotional facial expressions, execution of a facial movement, and experience of a disgusting taste. The three tasks were performed in the same specific order for all subjects to prevent influences from the facial movement execution (task 2) or the emotional experience (task 3) on the activity during the observation of emotional faces (task 1). Subjects were initially informed about the first task (see main text). After its completion, they were instructed about the other two tasks. Two subjects came back within two weeks for an extra visual or taste run due to technical problems.

Task 2: Movement of facial muscles

After task 1 (observation of dynamic facial expressions), subjects were trained on a motor task, which required them to pull up their noses as soon as a red cross on the screen changed to green. In the scanner, subjects performed 15 trials of the task. They contracted their facial muscles for the entire duration of the green cross (4 s) and relaxed them when the cross returned to red (16 s, baseline). The task instructions were given in pure motor terms ('pull up your nose') to avoid any explicit emotional connotations. However, the movement was selected to resemble the facial movements observed during a disgust response. The experience of disgust was induced by task three.

Task 3: Emotion experience

To repeatedly and reliably induce emotions in the MR environment, we delivered unpleasant and neutral liquids to the subjects. The unpleasant liquid consisted of a concentration of quinine diluted with sterile water as used in previous studies (Jabbi et al., 2007; Small et al., 2003). The neutral liquids consisted of artificial saliva diluted with sterile water in three different concentrations of 2.1%, 3.3%, and 5% (Farmachemie BV Haarlem, The Netherlands, art. nr. 39.701.130). During two taste runs, quinine and the concentration of saliva that the subject rated as the most neutral were delivered as a 0.5 cm3 bolus over a 5 s interval. Two CC of sterile water were delivered as a rinse at the end of each trial. The experimenter, who was standing next to the scanner, delivered the three types of liquids via three tubes that led to a pacifier which was placed in the mouth of the subject. Due to the length of the tubes (200 cm), the experimenter could keep distance from the subjects, which ensured that they were not distracted by her presence. Each taste run consisted of six quinine and six saliva trials presented in pseudorandom order to avoid the same condition from repeating itself more than twice in a row. In addition, the first condition of the first run was always different from the first condition of the second run. Two practice trials preceded the functional runs to ensure that subjects were comfortable with the task and able to swallow the liquids without moving their heads.

4.5.2 fMRI Analysis Control Tasks

Time series were high-pass filtered at 25 s for the motor task and 242 s for the taste task to remove low-frequency noise and slow drifts in the signal (the high-pass filter is based on the maximum time interval between two events of the same condition, which is very different between the tasks). For the experience task, tasting, rinsing and swallowing were modeled as separate conditions. Motion parameters were used as covariates in the motor and taste tasks, during which motion could be expected due to task demands. However, for the majority of subjects head motion never exceeded the acquired voxel size (3.5 mm). For one control subject we removed part of a run because of excessive motion. We checked whether our region of interest (ROI) had mirror properties (Figure 1c) by examining its activity in the control group (n=21) during facial expression execution (movement vs. baseline) and during emotion experience (disgust vs. baseline). The ROI was significantly activated in both cases (p<.001).

Magnetic resonance images acquisition

Scans were acquired using a 3T Phillips Intera Quaser (Best, The Netherlands) equipped with a synergy SENSE eight-channel head coil. Functional images were acquired using a T2*-weighted echo-planar sequence with 32 interleaved axial slices aligned with ac-pc, a thickness of 3.5 mm and no slice gap to cover the entire cortex (TR=1.5 s, TE = 28 ms, TA = 1.45 s, flip angle = 70 degrees). In addition, two T1-weighted anatomical images (1 x 1 x 1 mm) containing 160 slices were acquired parallel to the bicommissural plane.

Magnetic resonance images preprocessing

Data were preprocessed using the Statistical Parametric Mapping software package (SPM2, Wellcome Department of Cognitive Neurology, London, UK: http://www.fil.ion.ucl.ac.uk). Dummy scans, during which magnetization steady state was reached, were excluded from analysis. In addition, the first and last scans, where nothing was presented to the subjects, were excluded from analysis. Functional images from all sessions were corrected for slice timing (reference slice = 12, time bin=22) and subsequently realigned to the first volume of the first run to correct for shifts in head position. Because the experiment was done in two sessions (subjects went out of the scanner between Task 1 and the other two tasks to perform the motor and gustatory training), coregistration was performed in two steps. First, the subject's T1-weighted structural scan of the first experimental session was coregistered to the mean functional volume. Second, the anatomy of the second experimental session was coregistered to the first anatomy. This latter high-quality anatomy was segmented into gray matter, white matter and cerebrospinal fluid (CSF). The gray matter segment was normalized to a Montreal Neurological Institute (MNI) gray matter template and resulting parameters were used to normalize all functional images. Functional images were spatially smoothed with a 10 mm full-width half-maximum (FWHM) isotropic Gaussian kernel to improve the signal-to-noise ratio and to accommodate residual variations in functional neuroanatomy between subjects.

4.5.3 Eye Tracking

An infrared video camera (SMI, iView) was mounted onto the scanner bed to track subjects' gazes during the observation runs. The same video camera was also used to record facial movements during the motor task in order to confirm that all subjects pulled up their nose according to the instructions.

Eye tracking analysis

Because we used movies of facial expressions as stimuli, we defined dynamic ROIs in order to determine the time subjects spent looking at the face, the eyes only, and the rest of the face (face minus eyes). To this end, we first manually tracked for each movie frame the position of the pupils and mouth corners using a slowed-down version of the movies. We then used these moving coordinates to dynamically define an elliptical ROI around the face of the actors and a rectangular ROI covering both eyes (Figure S4). The face ROI not only takes horizontal and vertical displacement into account, but also follows the head tilts of the actor. The amount of time spent on the face was calculated by counting the number of samples falling within the face ROI during all movies. In addition, we counted separately the number of samples that fell within the eye region, and within the rest of the face (i.e. within the face ROI but outside of the eye ROI). We then correlated these two measures with age and the amount of activity in BA44 in the two groups separately.

Eye tracking results

Calibration errors or excessive noise prevented the analysis of the data of roughly half of the subjects. Stable eye tracking data were obtained and analyzed in 15 individuals with Autism Spectrum Disorder (ASD) and 10 typically developing (TD) subjects. There was no group difference in the time spent looking at various parts of our stimuli (face region, eye region, and face minus eyes region, all $p>.38$). This indicates that individuals with ASD and controls looked at the same parts of the tightly cropped dynamic facial expressions. We then examined whether changes in the way subjects look at the stimuli may account for part of the age-related changes in brain activity. We found that for both groups older subjects looked less at the eyes ($r=-.577$, $p=.003$), and more at the rest of the faces ($r=.633$, $p=.001$). This could be a beneficial strategy for our stimuli, because the lower part of the face often contained much information and movement (Figure 1a). Interestingly, we found that for the ASD group looking less at the eyes was accompanied by increased BA44 ROI activity (the correlation between the time spent looking at the eye region and BA44 activity was $r=-.54$, $p=.04$).

4.5.4 Control Experiment

Participants with schizophrenia

In addition to the 21 adult males with an autism spectrum disorder (age M=30.6, SD=10.09, IQ M=102.5, SD=14.81), and 21 typically developing males (age M=30.5, SD=9.85, IQ M=101.5, SD=17.40), 20 adult males with schizophrenia were recruited (age M=37.1, SD=10.53, IQ M=91.0,

SD=17.25) as part of a larger research project on the neurobiological basis of empathy. Individuals with schizophrenia and predominantly negative symptomatology were selected by experienced clinicians from a local mental health organization (Psychosencluster, GGZ Drenthe, Assen, The Netherlands). Diagnoses were confirmed by the administration of the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry (SCAN 2.1, Giel & Nienhuis, 1996). Current symptomatology was assessed by the Positive and Negative Syndrome Scale (PANSS, Kay, Fiszbein, & Opfer, 1987). The Social Functioning Scale (SFS, Birchwood et al., 1990) was used to assess the current level of social adjustment. All subjects with schizophrenia had normal or corrected-to-normal hearing and vision, were eligible for MRI research, and gave written informed consent to participate in the study, which was approved by the Institutional Review Board of the University Medical Center Groningen (METc).

Behavioral and fMRI analyses

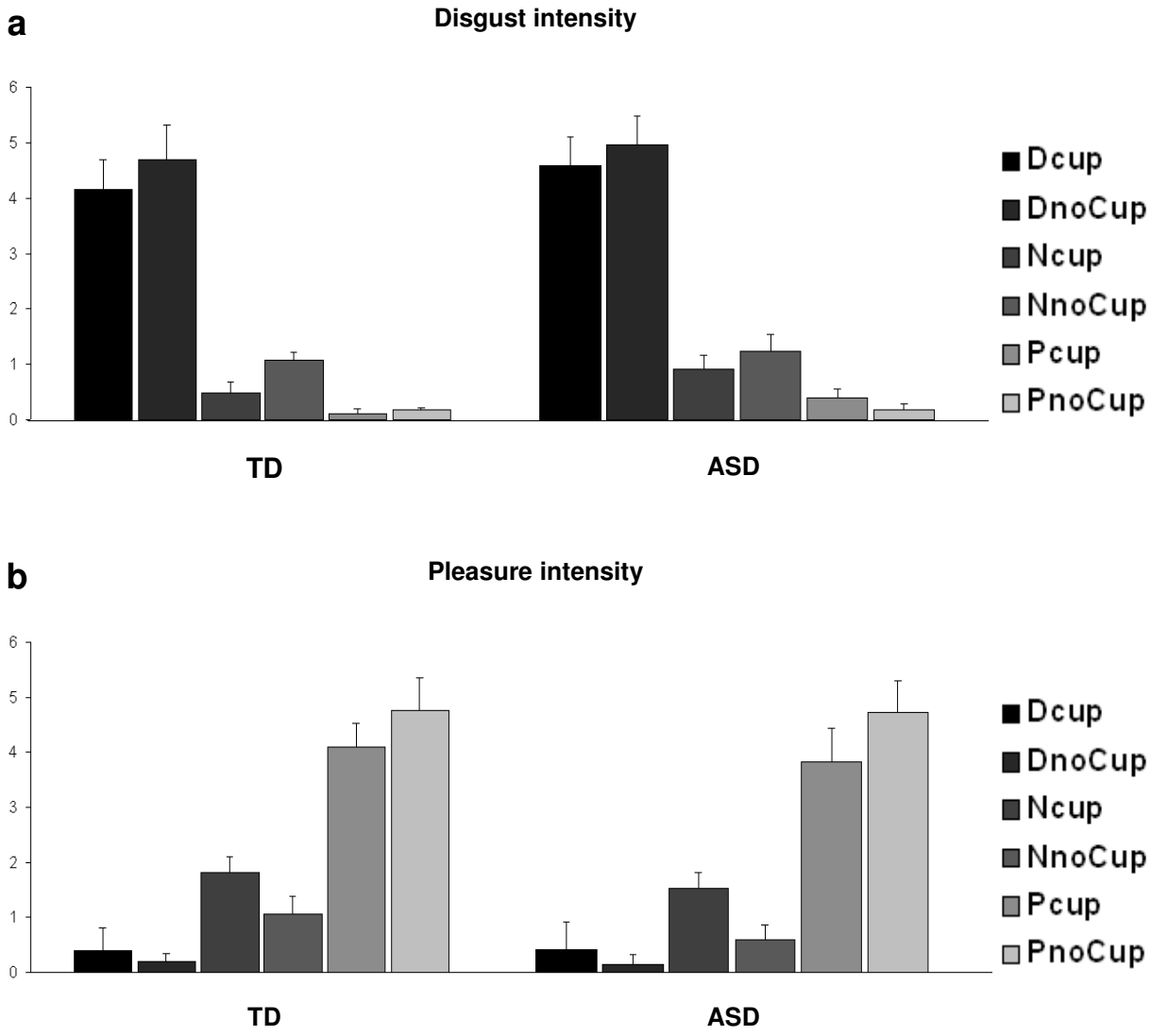
Participants with schizophrenia performed the same fMRI tasks and were given the same behavioral tests as the typically developing individuals and the individuals with ASD. For the SFS, 19 questionnaires were available for the 'client' version, and 15 for the 'other' version. Independent samples t-tests were performed to compare scores on the SFS in the group with schizophrenia and the group with ASD. MRI data preprocessing and subject-level analyses were performed in the exact same manner as described in the main paper for the other two groups. To compare activity in the BA44 ROI in the patient groups, we used a similar multiple regression analysis in MarsBaR as we did for the comparison between the ASD and TD group (two constants, two separate covariates to account for each group's IQ, and two for each group's age). To further examine the link between age, social adjustment, and brain activity that was found in ASD, we calculated the linear pairwise regressions within the group with schizophrenia and directly contrasted the regression slopes with those in the ASD group using a regression model including a constant for each group.

Results

The group with schizophrenia had SFS scores comparable to the ASD group (SFS-client: $T(36.0)=-.55$, $p=.59$, SFS-other: $T(32.3)=-1.21$, $p=.23$). In addition, mean activity in the BA44 ROI was not significantly different between the groups ($F=.04$, $p=.83$). In the group with schizophrenia, age and SFS scores were not positively related to each other nor to BA44 activity (Figure S3). A direct comparison between the slopes showed that the relations between age and BA44 activity, and age and SFS scores were significantly stronger in the ASD group (Figure S3).

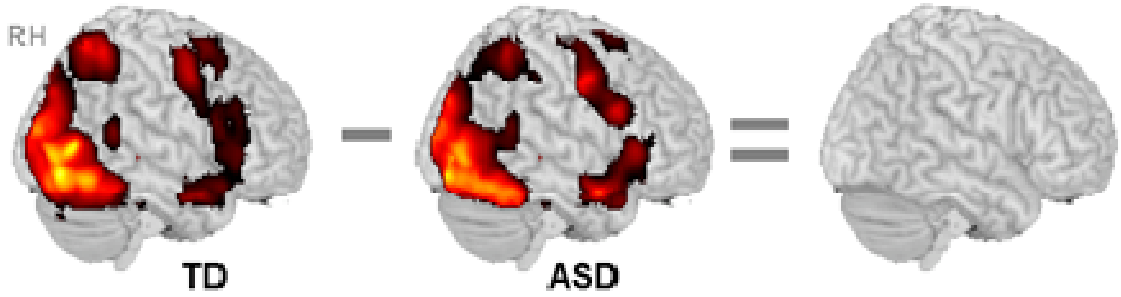
4.6 Supplementary Figures

Figure S1 Perceived intensity of disgust (a) and pleasure (b) as a function of movie and group.



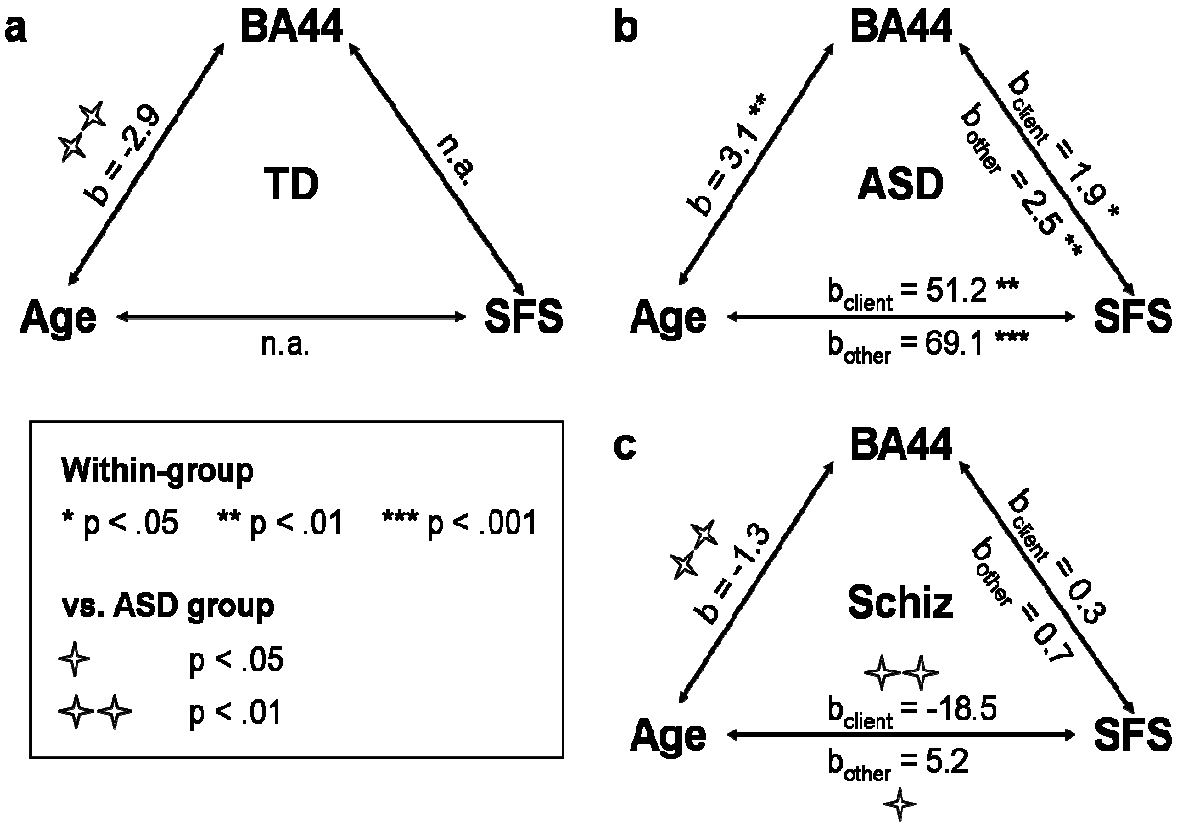
Error bars represent the standard error of the mean over subjects. After scanning, the subjects rated all movies on the perceived intensity of disgust and pleasure (0 to 6). A 3 Emotions (Disgust, Neutral, Pleasure) x 2 Contexts (Cup vs. No Cup) x 2 Groups (ASD, TD) mixed MANOVA on these ratings confirmed that the different emotions were perceived differently ($F(4,37)= 314.8$, $p=.000$ for emotion). There was also a difference between groups in the MANOVA ($F(2,39)=3.9$, $p=.03$). Separate ANOVAs showed that subjects with ASD rated the movies slightly higher on disgust and lower on pleasure than the TDs. Apart from this slight negative bias for the ASD group, there was no significant interaction between Group and either Emotion or Context for the disgust and pleasure ratings (all $p>.29$). This means the relative differences between the movies were perceived similarly in the groups. ASD, Autism Spectrum Disorder group; D, disgust; N, neutral; P, pleasure; TD, typically developing group.

Figure S2 Renders of the activity during the observation of emotional facial expressions.



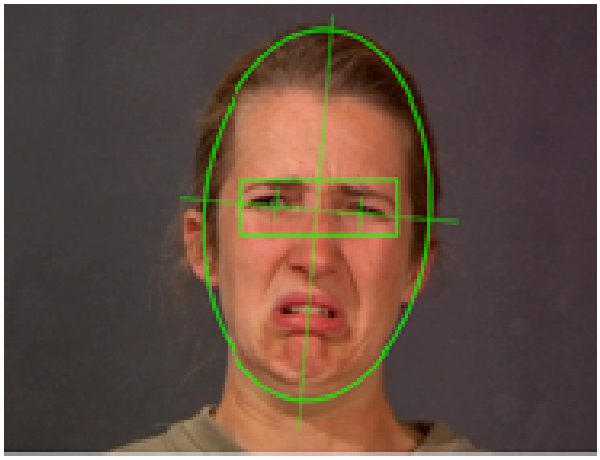
Faces (- baseline): TD and ASD group analysis, T=3.55, uncorrected p<.001, k>20, and between-group comparison TD >ASD (blue), T=3.33, uncorrected p<.001, k>20. All results for the TD and ASD group survive FDR<.05. Results are presented on a standard MNI brain. ASD, Autism Spectrum Disorder group; FDR, false discovery rate; MNI, Montreal Neurological Institute; RH, right hemisphere; TD, typically developing group.

Figure S3 Regression diagram



The diagrams show linear pairwise regressions between BA44 activity, age and social functioning (if applicable) in the (a) TD group, (b) ASD group, and (c) schizophrenia group. Regression slopes b are expressed in arbitrary units (x 100) per year/ SFS point and are reported in combination with their respective significance levels (* p<.05, ** p<.01, *** p<.001). Regression slopes that are significantly different from the ASD group are marked by stars in panels (a) and (c). SFS-client refers to the questionnaire that was filled out by the subject, SFS-other refers to the version that was filled out by an informant (e.g. parent or caretaker). ASD, Autism Spectrum Disorder group; SFS, Social Functioning Scale; TD, typically developing group.

Figure S4 Movie regions of interest (ROIs).



Frame of a disgusted facial expression with in green the tilted elliptical face ROI and the rectangular eye ROI.

4.7 Supplementary Tables

Table S1 Subject demographics

TD	Age	IQ	SFS-client	SFS-other	ASD	Age	IQ	ADOS	SFS-client	SFS-other
1	18	88	122.9	124.1	1	18	101	(4+4)= 8	102.6	91.3
2	18	97	129.6	128.1	2	18	73	(4+11)= 15	119.2	110.9
3	20	69	128.5	124.2	3	22	93	(2+5)= 7	99.9	107.1
4	21	82	120.3	126.1	4	22	82	(3+13)= 16	95.9	94.4
5	22	85	124.2	128.4	5	22	113	(4+5)= 9	117.1	112.1
6	22	73	124.8	133.1	6	23	82	(3+4)= 7	102.1	101.6
7	24	108	122.7	123.1	7	25	105	(5+6)= 11	106.6	105.9
8	25	117	124.7	129.6	8	25	100	(1+4)= 5	123.4	120.4
9	26	117	125.1	125.9	9	26	100	(3+7)= 10	110.6	113
10	29	120	127.9	123.2	10	26	116	(2+7)= 9	102.6	93.9
11	29	113	126.5	127.6	11	26	108	(4+10)= 14	107.6	101.1
12	29	121	122.1	126.6	12	26	108	(3+5)= 8	117.4	115.3
13	30	92	124.9	122.9	13	28	100	(3+8)= 11	102.5	95.1
14	32	128	128.9	130.6	14	34	124	(5+7)= 12	119.9	110.7
15	33	100	126.7	127.2	15	37	116	(2+8)= 10	122.5	116.2
16	40	92	128.6	122.6	16	38	117	(3+6)= 9	114.1	120.4
17	42	125	132	130.2	17	41	92	(3+7)= 10	123.4	114.1
18	42	85	128.9	122.2	18	42	85	(5+11)= 16	100.6	112.5
19	43	114	126.6	128.5	19	44	133	(4+8)= 12	126.7	123.9
20	43	96	126.1	128.8	20	45	104	(5+8)= 13	115.1	116.2
21	53	109	128.9	128.3	21	54	101	(3+7)= 10	126.1	128.5

ADOS, Autism Diagnostic Observation Schedule; ASD, Autism Spectrum Disorder group; SFS, Social Functioning Scale; TD, typically developing group.

Table S2 Peaks of activity during the observation of emotional facial expressions for the TD group

(k)	T	H	X	Y	Z	Area	BA
39977	20.21	L/R	42	-52	-16	Fusiform gyrus	
	17.59	L/R	-46	-70	4	Middle/inferior/superior occipital gyrus	17/18/19
	13	L/R	-16	-84	-16	Cerebellum	
	12.1	L/R	34	-52	58	Inferior parietal lobule	
	11.15	L/R	22	-26	-4	Hippocampus	
	9.21	R	62	-44	10	Middle/superior temporal gyrus	
	8.14	L/R	56	32	4	Inferior frontal gyrus	44/45
	7.31	L/R	-24	-2	-26	Amygdala	
	6.77	L/R	48	18	-22	Temporal pole	
	5.79	L/R	-36	28	0	Insula (anterior)	
917	6.48	L/R	0	12	58	Precentral gyrus	6
252	6.67	L	-58	-46	12	Superior temporal gyrus	
77	4.65	R	60	-18	48	Postcentral gyrus	1/2

Faces – baseline: TD group. Cluster size (k) and MNI coordinates (mm). Uncorrected $p<.001$, T=3.55, k>20. All results survive FDR<.05. BA, Brodmann area; FDR, false discovery rate; H, hemisphere; L, left; MNI, Montreal Neurological Institute; R, right; TD, typically developing.

Table S3 Peaks of activity during the observation of emotional facial expressions for the ASD group

(k)	T	H	X	Y	Z	Area	BA
37505	15.74	L/R	4	-86	-8	Middle/inferior/superior occipital gyrus	17/18/19
	13.45	L/R	38	-42	-22	Fusiform gyrus	
	11.47	L/R	30	-74	-18	Cerebellum	
	10.04	L/R	58	-46	8	Superior temporal gyrus	
	9.68	L/R	20	-24	-2	Hippocampus	
	9.13	L/R	20	-6	-18	Amygdala	
	7.83	L/R	58	24	20	Inferior frontal gyrus	44/45
	7.05	L/R	36	-48	64	Superior/inferior parietal lobule	
	6.55	L/R	-36	26	2	Insula (anterior)	
	5.27	R	42	-28	56	Postcentral gyrus	1/2/3
	5.13	L/R	48	8	-26	Temporal pole	
2080	8.62	L	-42	-4	42	Precentral gyrus	
	7.67	L	-54	14	24	Inferior frontal gyrus	44
	5.22	L	-40	-12	67	Precentral gyrus	6
328	4.9	L/R	10	4	70	Precentral gyrus	6
82	4.82	L/R	4	-24	30	Middle cingulate gyrus	

Faces – baseline: ASD group. Cluster size (k) and MNI coordinates (mm). Uncorrected $p<.001$, T=3.55, k>20. All results survive FDR<.05. ASD, Autism Spectrum Disorder; BA, Brodmann area; FDR, false discovery rate; H, hemisphere; L, left; MNI, Montreal Neurological Institute; R, right.